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Protein in Rice as Influenced By Variety and Fertilizer Levels

By

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TABLE OF CONTENTS

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PROTEIN IN RICE AS INFLUENCED BY VARIETY AND FERTILIZER LEVELS

By Frances E. Sturgis, R. J. Miears, and R. K. Walker[1]

Rice is one of the important cereals produced in Louisiana. In spite of its almost world-wide production and use only scattered and incomplete data are available on the amount and nature of the proteins it contains. No information is at hand, furthermore, on the genetic factors involved in protein content and composition of the various varieties. A large amount of data is available on the relation of soil type and fertilization to yields of rice, but no systematic study has been made on the effect of these factors upon protein content.

The International Rice Festival of 1950 featured rice as the most versatile of all foods. Certainly it is the most extensively grown food crop in the world. The total annual world production is approximately 3.3 billion 100-pound bags (27). Of this total, the United States produces somewhat over one per cent, of which 11 million bags is Louisiana's part. Louisiana produces 33 per cent of the American crop on 550 thousand acres and sells its production of rough rice for approximately 50 million dollars.

Rough rice is made up of approximately 21 per cent hulls and 79 per cent grain or hulled seed. The hulled seed contains from 6.4 to 9.4 per cent protein. The average is about 7.4 per cent because of increases in acreages in the low protein varieties such as Zenith, Bluebonnet, and Magnolia. Rexoro and Blue Rose are higher in protein, but of these varieties only Rexoro continues to hold up in acreage being planted. The generally recognized tables of Morrison's Feeds and Feeding give rough rice a protein content of 8.3 per cent and polished or milled rice a protein content of 7.4 per cent. The marketable protein of the Louisiana rice crop at present amounts to approximately 78 million pounds annually. If the average protein content were raised 1.0 per cent this would add approximately 10 million pounds of protein to Louisiana's production. When one considers the fact that Louisiana is producing approximately 41 million pounds of protein in beef while it is producing 78 million pounds in rice and that the beef protein sold for 45 million dollars while the rice proteins sold for approximately 3.8 million, it is very obvious that rice will continue to be in economic demand. Of course, not many Americans if given a free choice would substitute rice protein for beef protein, but this has been done largely in countries

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of low income levels, particularly in the Orient. The fact that it has been done for thousands of years argues for the quality of rice protein. It also suggests that possibly where rice makes up a large part of the daily diet primitive people without any scientific knowledge may have selected rice varieties of good protein content. Americans, on the other hand, in their all-out effort to breed crops for higher yields may be paying insufficient attention to protein quantity and quality. This has been done in hybrid corn breeding. Although rice is primarily a carbohydrate crop, one cannot overlook the fact that protein in rice sells from the farm for 5 cents per pound while it sells from the farm for 50 cents per pound in meats. It is also a fact that rice contains all the essential amino acids for growth and maintenance in man (16). Is it without some significance that Rexoro, the rice variety most in demand by the American consumers, is a high protein rice?

The purposes of this study have been (a) to determine the variation in protein content of certain varieties and new selections now being grown at the Louisiana Rice Experiment Station, (b) to determine the variations in protein distribution in solubility fractions as related to variety or new genetic selections, and (c) to determine the effects of different fertilizer levels on protein production.

REVIEW OF LITERATURE

Although rice (*Oryza sativa*) ranks first in production among the grain crops of the world, its study is greatly in need of organization. Compared with the proteins of wheat, corn and barley, those of rice have been but little studied. Unlike the proteins of other cereals, a greater percentage of the proteins of rice belong to the rather uncommon class of proteins called glutelins. A large portion of the data in the literature on rice has been concerned with the total alkali-soluble proteins. The protein of rice soluble only in dilute alkali was first isolated by Rosenheim and Kajiura (38) in 1908 and named oryzenin. Based on solubility, there are four types of proteins in rice (52). They are water-soluble albumin, salt-soluble globulin, alcohol-soluble prolamin, and dilute-alkali-soluble glutelin.

Rice contains less protein than the other top-ranking cereal grains, wheat and corn. When, however, the proteins of rice, wheat and corn were compared in feeding studies at the same level of intake, rice protein had a higher biological value than the proteins of wheat or corn (44). Sure and House (46) determined by the nitrogen-balance method the relative biological values of proteins in cererals fed at a 5 per cent protein level. The protein utilization for the milled cereal grains was: rice 75.1, wheat 60.0, rye 63.1, and corn 32.0, with 100 as a basis of comparison. The findings for the whole grain were: rice 80.0, wheat 76.1, rye 73.2, corn 78.8, and rolled oats 75.6. Mitchell (26) also found that the biological value of brown rice protein was superior to that of corn or oats on the same level of intake (5 per cent). Workers at the Connecticut Agricultural Experiment Station (32) compared rice protein with the pro-

tein of wheat and maize and found that the rice proteins yielded relatively high amounts of the basic amino acids: arginine, histidine, and lysine, and comparatively little ammonia and non-amino nitrogen.

Price (36) determined arginine, lysine and valine contents of four different varieties of wheat by microbiological methods. Results indicated that the wheat lowest in total nitrogen contained a higher proportion of these amino acids than varieties highest in nitrogen. This suggests qualitative as well as quantitative differences in protein content of wheat varieties.

In its general amino acid make-up, rice protein more closely resembles the majority of animal proteins than do those of wheat or maize. This may explain the extensive use of rice as an almost exclusive diet for millions of people in the Orient in spite of its low protein content.

Block and Bolling (4) have reported the following values in per cent for the different amino acids in rice protein: arginine 7.2, lysine 3.2, tyrosine 5.6, tryptophane 1.3, histidine 1.5, isoleucine 5.1, phenylalanine 6.3, cystine 1.4, methionine 3.4, threonine 3.9, leucine 9.0, and valine 6.4. The biological value of a protein seems to depend, in general, upon the contents of these dietary essential amino acids.

The protein content of rice varies from 6.0 to 9.0 per cent and is affected by many factors including genetic variety. The hope and belief that the protein content of rice might be increased considerably by proper breeding is worthy of serious consideration. Seeds of closely related species resemble each other in the character and proportions of the several different forms of proteins which they contain, e.g., the seeds of cereals are the only ones containing proteins soluble in strong alcohol (30). Therefore, since little work has been reported on variation of protein content with different rice varieties, it is pertinent to review the work that has been reported on variation of other grain proteins with variety.

In a study of the influence of heredity on protein content of wheat, Shutt (43) found significant differences in the averages of protein contents of 165 samples, representing 45 varieties.

Multiple factor inheritance of crude protein content was indicated from crosses among three varieties of wheat (53). Clark (6) also working on breeding wheat for higher protein content showed that the inheritance of crude protein content is as complex as that of yield, and that environment is fully as important in determining the result in one case as in the other. Strains with a crude protein content higher than that of the better parent were not obtained. These inheritance studies indicated that the total amount of crude protein per acre, however, may be increased through improvement in yield with maintenance of the crude protein content of the highest parent. To increase the crude protein content of the grain materially it appears necessary to select a high protein parent even at a sacrifice in yield.

Using 15 corn hybrids and varieties grown at two nitrogen levels in

1946 and 18 hybrids at three nitrogen levels in 1947, Viets and Domingo (50) showed significant differences in nitrogen content and yields.

Miller, Aurand, and Flach (25) analyzed nine different single crosses of inbred lines of yellow corn for lysine, tryptophane, and methionine. Individual replicates of these varied in crude protein content from 8.48 to 14.13 per cent. Amounts of each of the amino acids studied varied directly with the crude protein content; consequently, the quality of the protein as measured by these amino acids was not changed within the range of protein covered.

With studies of inheritance in some summer and winter wheat crosses of the F_4, F_5, and F_6 generations Swen (47) showed that the protein content was inheritable but strongly influenced by weather and soil conditions. In several crosses, Swen found that the protein content of the progeny actually was as much as 1.4 per cent greater than that of the highest parent. He found no complete relation between protein content and yield of grain.

Investigations on the separation by solubility of the proteins of rice and other seeds give evidence that the protein fractions vary with the variety and with the total protein content.

Sadasivan and Sreenivasan (40) found, on analyzing rice varieties having different morphological characteristics, that colored and coarse-grained varieties of rice usually contain greater percentages of protein and minerals than do the fine-grained varieties. Sreenivasan also (44) reported that the poor quality of rice as ordinarily consumed in India is largely traceable to the choice of the wrong varieties as well as to the refining processes. He conducted a rat-feeding study on six varieties of Indian rice and found that the gain in weight was considerably different for the different varieties. It was also shown that the same percentage of rice protein from two different varieties caused significant differences in rate of growth, thus showing that the quality as well as the quantity of protein in rice varies with variety.

Basu and Basak (3) observed that the two most common varieties of Indian rice, Aman and Aus, were different in nitrogen distribution and solubility fractions as indicated:

	Water Extract	5% NaCl Extract	75% Alcohol Extract	0.4% Alkali Extract	Total Recovery %
Aman	5.8	22.6	3.7	62.7	94.8
Aus	7.5	29.2	3.0	55.7	95.4

The globulins (salt-soluble) and the glutelins (alkali-soluble) of both varieties were analyzed by the Van Slyke method and were found to differ in nitrogen distribution. The Aman rice contained more sulfur-containing amino acids and more arginine than the Aus rice.

Using the procedure devised by Csonka (8), Kik (20) analyzed the proteins of rice and rice by-products by extracting with four solvents:

1 per cent sodium chloride, 60 per cent alcohol, 0.1 per cent alkali, and an acid alcohol mixture. The distribution for the different rice samples was as follows:

Solvent	Whole rice %	White rice %	Rice from S treated plot %	Rice from Check plot %
1% NaCl	13.81	11.50	16.20	14.53
60% alcohol	3.66	5.75	4.00	3.00
0.1% alkali	44.22	40.85	40.60	40.00
acid alcohol	3.98	5.33	4.70	3.70
residue	25.90	27.60	28.50	32.23
% Nitrogen Extracted	91.58	90.50	93.40	93.23

The relative amounts of the different proteins in high- and low-protein corn were studied by Miller, Aurand, and Flach (25), and it was indicated that a considerably larger part of the protein is present as glutelins and globulins in high-nitrogen corn than in low-nitrogen corn, and that these had been formed at the expense of the amides and albumins. He found also that the glutelin fraction varied the most from the high- to the low-protein corn. The quantity of the various proteins was correlated with the total nitrogen content.

Anderson and Ayre (1) showed that the distribution of nitrogen in barley is influenced by variety. They found that at any given total nitrogen content, the proportions of each protein fraction may differ from one variety to another. Within and between varieties there was a highly significant positive correlation between alcohol-soluble protein nitrogen and total nitrogen. With increasing total nitrogen the proportion of salt-soluble fraction decreased, the alcohol-soluble increased, and the insoluble fraction remained relatively constant. The insoluble fraction was defined as that protein not soluble in salt or alcohol solutions.

It has been found that the extraction of albumins and globulins of barley is most satisfactory with 5.0-6.5 per cent K_2SO_4 or 7 per cent NaCl (21). Urion, Lejeune, and Collin (49) found that concentrations higher than the above-mentioned extracted a small part of the gluteline. None of the salt solutions extracted any of the prolamins, but the extraction of this alcohol-soluble fraction was made easier by first extracting the barley with saline solution. Osborne (31) also concluded from his data on barley and wheat flours that alcohol extractions should be preceded by a saline extraction since alcohol will dissolve part of the albumins and globulins.

During ripening of rice the total nitrogen content was shown by Tadokoro and Abe (48) to exhibit no definite tendency to change, but the ratio of protein to non-protein nitrogen increased constantly. The

water-soluble group decreased and the salt-soluble was inconsistent in its changes. The rice samples were harvested at four weekly intervals, the last harvest consisting of completely ripe samples.

The average farmer is usually more interested in the yields of his crop than the quality of the product, and he therefore uses a top-dressing of nitrogen fertilizer to increase the yield. There is ample evidence that increased yields alone do not represent the full and true value of nitrogen fertilizer for small grain crops. Experimental results are reported in which nitrogen fertilizer increased the protein content of small grain from 12 to 55 per cent. In general, protein content cannot be increased much over 25 per cent without decreasing the yield; also if stands are too thick, yields are increased at the expense of protein. Earley and De Turk (10) present evidence that points to the conclusion that the application of enough nitrogen to produce maximum yields of corn will also assure a higher protein content if the rate of planting is limited.

It is, of course, generally recognized that the composition of a crop is affected by the fertility of the soil. From a practical standpoint, however, it is not enough to know that such a relationship exists. Quantitative information must be available if the crop growers are to be properly guided in their fertilizer programs and farming operations. How much change in the composition of crops which can be brought about by fertilizer or increases in soil fertility must be known. One must know also how changes in plant composition are related to increase in crop yield. Information on these problems in connection with wheat, barley and oats emphasizes the fact that the changes in the protein content of small grain are related to the increase in crop yields resulting from fertilization.

Pierre (35) found that, in general, the greater the increase in yield from a given amount of nitrogen fertilizer the less is the change in protein content of the crop. The corollary is likewise true. When the soil is very deficient in nitrogen and when the amount of nitrogen fertilizer added is small or insufficient to obtain maximum yields, most or all of the nitrogen absorbed by the plant is utilized in increasing yields. In some cases, the percentage protein is actually lowered, because the crop utilizes the small amount of nitrogen added during early growth. During the latter part of the season, therefore, when the grain is forming, less nitrogen is available in relation to the needs of the crop than where no nitrogen is applied. At the other extreme are the soils that are already well supplied with nitrogen or to which large amounts of nitrogen fertilizer are added in relation to the needs of the crop. In some cases luxury consumption results. The plant continues to take up nitrogen beyond normal requirements, and since little or none of it is used for increased carbohydrate yield the protein percentage in the plant increases but the total yield is low.

A situation encountered quite often in fertilizer practice involves a soil quite deficient in nitrogen and where the amount of nitrogen added

is about sufficient to meet the needs of the crop and to produce maximum or near maximum yields. Under such conditions one usually gets both an increase in crop yields and an increase in the protein content of the crop. The increases in protein content and grain, however, are usually small and in the order of 10 to 25 per cent.

Many investigations have been designed to demonstrate the effect of available nitrogen on the protein content of various crops. The performance of complete fertilizers, potash and phosphoric acid have also been considered.

The effect of nitrogen fertilizer on the yield and protein content of wheat varieties was reported by Peterson (34). In some cases the fertilizer is utilized in increasing the yield and hence there is no material difference in the protein content of the grain. He found that only the early applications of nitrogen induced significant increases in protein content. When an effect was obtained, a heavier nitrogen application induced a greater percentage protein and a greater yield.

Mosolov (28) found that early and medium maturing wheat varieties compared with late maturing varieties used relatively small quantities of nutrients and utilized these more effectively for the formation of grain of high protein content. Nitrogen and phosphoric acid when applied to the early and medium maturing varieties helped avoid early aging of leaves and of the plant as a whole.

Increases in protein content of corn of 7.8 to 9.4 per cent in a dry year and of 5.5 to 8.2 per cent in a wet year through nitrogen fertilization on a poor soil were reported by Kohnke and Vestal (22). They found that corn from an inherently more fertile soil had a higher protein content and caused more growth in a hog-feeding test than corn from an inherently poor but highly fertilized soil. Viets and Domingo (50) also found that the protein content of corn could be appreciably increased by the use of nitrogen fertilizer. Even on loamy sand the recovery of fertilizer in the grain was 48 per cent of the nitrogen applied.

Gardiner (11) found that with several varieties of wheat whose protein content varied from 9.6 to 10 per cent and averaged 9.9 per cent the increased protein content due to 150 pounds per acre of ammonium sulfate top-dressing averaged 0.36 per cent. He found the average recovery of the applied nitrogen was 26 per cent in the grain.

The addition of mineral nitrogen increases the yield and protein content of crops under favorable conditions. The effectiveness of the mineral nitrogen as a fertilizer, however, depends on seasonal and soil conditions and on the amounts of nitrogen applied. In order to obtain the best response, nitrogen fertilizer must be balanced with the proper amounts of phosphorus and potassium.

Plot trials were conducted by Schmitt and Schneis (41) to determine the increase in protein content of grain effected by a late application of nitrogen fertilizer. The grains tested were barley, oats, and

spring wheat. It was found that the addition of sodium nitrate just before heading caused a marked increase in percentage of protein when compared with samples from plots for which all the nitrogen was added at the time of seeding.

Selke (42) conducted field experiments as well as pot experiments for four years to prove the value of nitrogen applied at a late stage in growth to increase the nitrogen content of the common grains as well as yields and quality. He showed that the increased nitrogen was present as protein in the grain. He also found that the degree of effectiveness of the late top-dressing was dependent upon the moisture supply and that the application should follow a good rain.

That the percentage of nitrogen in wheat was higher when the soil had received a nitrogenous fertilizer and that it increased with the quantity of nitrogen added was demonstrated by Demolon (9).

McCalla and Woodford (24) and also Ginsberg (12) reported that limiting the supply of potassium for wheat plants grown with an otherwise complete fertilizer resulted in a decreased nitrogen content. Reduced potassium supply also resulted in an increase in the proportion of grain to total yield which was partially caused by retarded growth. Byczkowski and Jarmusz (5) reported that increase in potassium fertilizer together with a moderate supply of nitrogen exerted no influence on the content of nitrogen in barley. With a higher nitrogen supply, however, an application of potassium lowered the nitrogen content of the grain. Joret and Malterre (19), furthermore, related that the nitrogen content of wheat underwent very slight change with increasing doses of potassium in the presence of a fixed amount of nitrogen. It has been reported by Hibbard and Grigsby (14) that increasing the potassium fertilizer applied to grain had no effect on the nitrogen content.

Reitz and Myers (37) found that the application of superphosphate fertilization on wheat increased yields of grain and straw and decreased the percentage of protein. Nehring (29) reported that added phosphate had a beneficial effect only if the water supply was adequate. The phosphate decreased the nitrogen content but increased the yield so that the over-all effect was to increase the protein yield.

The protein content of rice can be increased by as much as 20 per cent by suitable fertilization up to the time of flowering of the plants, according to Sadasivan and Sreenivasan (40).

Annotel (2) showed that calcium phosphate applied to rice fields gave a marked increase in the protein content of the grain.

The protein content was highest from the nitrogen and complete fertilizer plots. High protein was associated with low carbohydrate content. Field experiments with rice receiving different fertilizer treatments including ammonium sulfate, calcium phosphate, and potassium chloride individually and as a complete mixture were described by Pain (33).

In a controlled study of rice in pots, Sturgis and Reed (45) found that the yield of rice on a nitrogen deficient soil could be increased 25

per cent by use of complete fertilizer. The increase in the protein content was only 10 per cent. The protein content of rice on a naturally fertile soil was 39 per cent higher than on a deficient soil.

Studies on the effects of increasing the nitrogen supply on yields and composition of corn, rice and wheat have been reported by Russell (39). If the nitrogen fertilizer greatly stimulates crop growth, the nitrogen content expressed as percentage of dry weight will often decrease although the total yield increases. This is because the extra growth which the added nitrogen stimulates the plant to produce has lower nitrogen or protein content than that produced by a nitrogen starved crop. As the level of nitrogen supply increases, the stimulation on total growth becomes smaller so the uptake of fertilizer nitrogen is increasing relative to the additional production resulting from it. The nitrogen content of the crop begins to rise, but only becomes large when the growth of the crop ceases to respond economically to the additional fertilizer. The effect of added nitrogen on protein content is most effective when applied near harvest. The reaction of rice was found to be intermediate between corn and wheat.

MATERIALS AND METHODS

I. Materials

Rice grown in two different years was used to determine the effects of various fertilizer levels on protein production. The rice was grown at the Rice Experiment Station, Crowley, Louisiana, on Crowley silt loam soil. Nitrogen fertilizer was applied as ammonium nitrate, which is 32 per cent nitrogen; phosphorous as superphosphate or calcium phosphate, which is 20 per cent P_2O_5; and potassium as potassium chloride, which is 50 per cent K_2O. These three fertilizer factors were varied

TABLE I

Fertilizer Treatments for 1949 and 1950

1949	1950	
N-P_2O_5-K_2O	N-P_2O_5-K_2O	N-P_2O_5-K_2O
lbs./A.	lbs./A.	lbs./A.
0-0-0	0-0-0	20-20-40
0-18-12	0-0-20	20-40-0
0-36-24	0-0-40	20-40-20
0-54-36	0-20-0	20-40-40
12-18-12	0-20-20	40-0-0
24-36-24	0-20-40	40-0-20
36-54-36	0-40-0	40-0-40
18-18-12	0-40-20	40-20-0
36-36-24	0-40-40	40-20-20
54-54-36	20-0-0	40-20-40
	20-0-20	40-40-0
	20-0-40	40-40-20
	20-20-0	40-40-40
	20-20-20	

to determine their effects individually and in different combinations. The fertilizer was applied at time of seeding with a seed and grain drill. This method of application has been found to increase the effectiveness of the fertilizer by effecting a better weed control and a closer contact of the rice plant roots and fertilizer (51). In Table I the fertilizer levels are listed for both years.

The 1949 fertilizer treatments were replicated three times; the protein yields were determined separately for the three replicates. In 1950 the replicates were combined and analyses were made on one representative sample for each fertilizer level. The yield values reported for the

TABLE II

Characteristics of Varieties and Selections

Sample No.	Variety or Selection	Avg. Date Maturity	Hull Color	Pubescence	Ease of Threshing	Grain Type*	Table Quality**
47	Zenith	8/24	straw	hairy	Dif.	M.	M.S.
37	Bluebonnet	9/9	straw	smooth	easy	L.S.	F.
50	Dwarf Texas Patna	9/18	gold	smooth	easy	L.	F.
38	322A6-23	9/13	straw	hairy	easy	M.L.	F.
51	Lacrosse	9/16	straw	smooth	Dif.	M.	S.
32	Arkrose	9/16	straw	hairy	Dif.	M.	S.
31	Magnolia	8/23	straw	smooth	easy	M.L.	F
62	Nira	9/18	straw	smooth	easy	L.L.	F.
42	31-9-2-4	10/1	straw	smooth	Dif.	M.	S.
36	Century	8/31	gold	smooth	easy	L.	-***
35	Fortuna	9/14	straw	hairy	easy	L.L.	F.
59	7/8 Rexoro	10/9	gold	smooth	Dif.	L.S.	F.
58	4II-1-8	9/9	straw	smooth	easy	L.S.	F.
61	3-72-3	10/2	gold	smooth	easy	M.L.	F
39	Prelude	9/2	straw	hairy	Dif.	L.L.	S.
43	Caloro	9/8	straw	hairy	Dif.	P.	S.
44	Improved Blue Rose	9/24	straw	hairy	Dif.	M.	S.
34	7/8 Rexoro-3-12	10/11	gold	smooth	Dif.	L.L.	F.
60	Purple Bran	-***	straw	smooth	-***	M.	-***
33	6I-25-12	10/4	straw	hairy	easy	M.	S.
56	Rex-Nira	9/18	gold	smooth	-***	M.	S.
46	Rexoro x Purple Leaf	10/2	gold	smooth	easy	M.L.	F.
41	Rexoro-Delitus	10/5	gold	smooth	easy	L.	Sc.
52	4II-4-1-4	10/5	straw	smooth	Dif.	L.S.	-***
54	15/16 Rexoro-3-22	10/11	gold	smooth	easy	L.S.	F.
45	Blue Rose 41	9/24	straw	hairy	Dif.	M.	S.
57	7/8 Rexoro-3-22	10/9	gold	smooth	Dif.	L.S.	F.
55	Texas Patna x Rexoro	10/5	gold	smooth	easy	L.L.	F.
40	Rexoro	10/11	gold	smooth	easy	L.S.	F.

*M.—medium, M.L.—medium long, L.—long, L.L.—large long, L.S.—long slender P.—pearl.
**F.—flaky, S.—sticky, M.S.—medium sticky, Sc.—scented.
***No data available.

1950 rice samples are averages for all replicates grown for each treatment.

To determine the variation in protein content of certain varieties and new selections, and to determine the variation in protein distri-

bution in five solubility fractions as related to variety or new genetic selections, twenty-nine varieties and new selections were analyzed for total protein nitrogen, water-soluble albumin, salt-soluble globulin, alcohol-soluble prolamin, alkali-soluble glutelin, and residual or insoluble protein. All varieties and selections were grown under the same soil and seasonal conditions. The varieties and their characteristics are shown in Table II.

II. Methods

A. Preparation of Rice Samples

Air-dried rough rice samples were hulled by means of a Smith shelling device as approved by the U.S.D.A., Production and Marketing Administration, Grain Branch. The hulled rice grains were then separated from the loose hulls by means of a Bates laboratory aspirator. This method was consistent in that each time 50 grams of rough rice were hulled and separated, approximately 9.7 grams of hulls and 39.3 grams of grains were obtained. A Wiley mill was used to grind the hulled grain to pass through a 20-mesh sieve. Rice samples for all analyses were prepared in this manner. Moisture determinations were made on all samples. The macro-Kjeldahl method was used for the determination of protein in the residue and total protein content of all samples. Proteins of the varieties and selections were fractionated by extracting with four solvents: distilled water, 5 per cent sodium chloride, 70 per cent ethyl alcohol, and 0.2 per cent sodium hydroxide. Micro-Kjeldahl determinations were run on these four fractions.

B. Protein Separation

The mthod for protein fractionation is a modified combination of the methods reported by Csonka (8) and by Lund and Sandstorm (23). Modifications made were shown by experimental verification to give the most reliable results.

Five grams (5.0000) of ground rice were weighed into a 500-ml Erlenmeyer flask. The rice was defatted by three half-hour extractions with 25 to 30 ml of dry diethyl ether. The extractions were carried out by allowing the sample to stand in the ether for 30 minutes with occasional shaking. After 30 minutes the ether was decanted, and the sample was extracted with another 25 to 30 ml of ether. After the third ether extraction, the sample was left unstopped for a short time so that the ether which could not be decanted would evaporate.

The first protein that was separated from the rice was the water-soluble albumin. Albumins were extracted by three water extractions of 30 to 50 ml of distilled water each. Two of the extractions were for one hour and the third was overnight. After the sample had remained in the water for the desired length of time, the sample was filtered on Whatman No. 1 filter paper. Solid material that was caught by the filter paper was washed with the solvent into the 500 ml Erlenmeyer flask con-

taining the sample. After the third extraction the residue was washed with two 10 ml portions of water. The filtrates from the three extractions and the two washings were combined and diluted to volume in a 200 ml volumetric flask. A 50 ml portion of this volume was taken for a micro-Kjeldahl determination for nitrogen.

After the albumin separation the same five-gram rice sample was extracted with 5 per cent sodium chloride to separate the globulin from the rice. The method for the 5 per cent sodium chloride extraction was the same as that for the water extraction.

The filtrates and washings were combined and diluted to 200 ml in a volumetric flask. A 25 ml portion of this volume was taken for a micro-Kjeldahl nitrogen determination.

The residue was extracted next with a 70 per cent ethyl alcohol solution to remove the prolamin, using the same procedure that was used for both water and salt extractions.

The three filtrates and two washings were combined and diluted to 250 ml in a volumetric flask. Fifty ml of this volume was taken for a micro-Kjeldahl nitrogen determination.

The fourth and last extraction was with 0.2 per cent sodium hydroxide to remove the oryzenin or rice glutelin. The extracting, filtering, and washing procedure employed for the three previous extractions was altered only in that there were four extractions with 0.2 per cent sodium hydroxide, three of two hours each and a fourth overnight.

After the second 10 ml washing, all of the residue was washed from the Erlenmeyer flask onto the filter paper with 0.2 per cent sodium hydroxide. This was allowed to filter overnight and was designated as the third washing. The four filtrates and the three washings were diluted to volume in a 500 ml volumetric flask. A 25 ml portion of this volume was used for the micro-Kjeldahl nitrogen determination. The residue on the filter paper was denoted as insoluble protein and was analyzed for nitrogen by the macro-Kjeldahl method.

C. Micro-Kjeldahl Determination

Micro-Kjeldahl determinations were made on all protein solubility fractions of the variety samples. An aliquot of the protein solution was pipetted into a 100 ml Kjeldahl flask. The aliquot used was determined by the anticipated concentration of nitrogen in the fraction being analyzed, i.e., 50 ml of the water and alcohol fractions and 25 ml of the sodium chloride and sodium hydroxide fractions. One gram of nitrogen-free potassium sulfate and five drops of 5 per cent cupric sulfate were added to the sample in the Kjeldahl flask. Two ml of concentrated sulfuric acid (sp. gr. 1.84) were pipetted into the flask, and lastly, a glass bead was put in the flask to facilitate boiling and to prevent bumping.

The flask containing the sample was heated gently over a Bunsen burner to evaporate most of the solution. When the contents of the flask were concentrated to 15 to 20 ml, the heat was increased and the

heating was continued a few minutes after all carbonaceous material was digested and only the clear green solution remained. The flasks were cooled to 40-50° C and diluted with 10 ml distilled water while being shaken.

The distillation was carried out with a Pregl type (A. H. Thomas) micro-Kjeldahl steam distillation apparatus. Employing a 125 ml Erlenmeyer flask as a receiver, the ammonia distilled from the samples was caught in 2 ml of standardized 0.1N sulfuric acid and 10 ml of distilled water contained in the receiving flask. The delivery tube into the receiving flask was held below the surface of the liquid in the flask.

The digested contents of the Kjeldahl flask were poured into the distilling flask of the apparatus. Approximately 10 to 15 ml of distilled water were used to rinse the flask and funnel. Eight to ten ml of 50 per cent carbonate-free sodium hydroxide were added to the distilling flask and the sample was steam distilled until approximately 50 ml of distillate were collected. The receiving flask was then disconnected and the delivery tube was rinsed with 3 to 5 ml of distilled water; the rinsings were caught in the receiving flask.

The contents of the receiving flask were quantitatively transferred to a 100 ml volumetric flask and diluted with distilled water to approximately 80 ml. Ten ml of Nessler's reagent, prepared according to directions in Hawk, Oser, and Summerson (13), were added and the flask was filled to the mark with distilled water. The color developed was read in a Beckman DU quartz spectrophotometer at 480 uu wavelength and a slit width of 0.3.

Six standard ammonium chloride solutions were prepared containing 0.1, 0.2, 0.5, 1.0, 1.5 and 2.5 mg. of nitrogen, respectively. Optical density was read after Nesselerization in the Beckman DU spectrophotometer and standard curves were plotted. Milligrams of nitrogen in the rice samples were determined from their corresponding optical densities by means of these standard curves.

D. Macro-Kjeldahl Determinations

Total protein of all samples and insoluble protein of the varieties and selections were determined by the following procedure. The analyses were made on a Precision Scientific Co. Kjeldahl apparatus.

1. **Digestion:** The method consisted of weighing accurately 1.2-1.4 grams of sample onto a sheet of Whatman No. 42 ashless filter paper. The filter paper was carefully folded around the sample so that it could be dropped into an 800 ml Kjeldahl flask allowing none of the sample to touch the neck of the flask. An oxidizing mixture of 15 g of nitrogen-free potassium sulfate, 0.6 to 0.8 g cupric sulfate ($CuSo_4 \bullet 5H_2O$) and 20 ml of concentrated sulfuric acid (sp. gr. 1.84) were added to the Kjeldahl flask in the order stated.

The Kjeldahl flask containing the sample and oxidizing mixture was then placed on the digestion rack. The rheostat was set at 20 to begin the digestion, but when frothing began the heat was increased to

maintain a vigorous frothing. The flask was rotated frequently and the heat was carefully controlled to prevent the frothing mixture from entering the neck of the flask. By the end of the digestion the heat had been increased to a full rheostat reading of 100. Heating was continued at full heat for 30 minutes after all the black material had disappeared, i.e., after all carbonaceous material had been digested.

The heat was turned off at the end of 30 minutes and the flask was allowed to cool on the digestion rack to 40°-50° C. After the flask was cooled it was removed from the digestion rack.

The samples were diluted with 250 ml of ammonia-free distilled water. The water was added with shaking of the flask to avoid crystalization of the sample.

2. **Distillation:** Fifty ml of saturated boric acid solution were measured into a 500 ml Erlenmeyer flask employed as a receiving flask. The receiving flask was then connected to the delivery tube of the distillation rack and the water in the condensers was turned on in preparation for the distillation.

When the Kjeldahl flask was cool, 75 ml of 50 per cent carbonate-free sodium hydroxide were added to the flask held at a 30° angle in order that the sodium hydroxide would collect in a layer beneath the sulfuric acid layer. The layers were not allowed to mix. One to two grams of 20-mesh zinc metal were added to the flask, and the flask was quickly connected to the distilling rack. The heat was turned on with the rheostat set at 100. Then with the stopper held firmly in place, the flask was shaken gently until the sulfuric acid layer was neutralized as indicated by the deep blue color of cupric ammonium sulfate. When the sulfuric acid was neutralized, the flask was shaken vigorously to mix completely the two layers. The flask was then put on the burner and the mixture was distilled at a smooth steady rate to collect 100 ml of distillate over a period of 45 to 60 minutes. The receiving flask was disconnected from the delivery tube and the delivery tube was rinsed with 3 to 5 ml of distilled water; rinsings were caught in the receiving flask.

The ammonia in the boric acid was titrated with standardized sulfuric acid (one-tenth normal) using 3 to 4 drops of methyl red as the indicator solution.

E. Moisture Determinations

Moisture content was determined on accurately weighed two-gram samples which were heated to constant weight in an oven at 106° C.

RESULTS AND DISCUSSION

In Tables III through IX, given below, are reported the results of experiments conducted to determine the effects of different fertilizer levels on protein production, and to determine the variation in protein content and protein distribution of certain varieties and new selections now being grown at the Louisiana Rice Experiment Station, Crowley, Louisiana.

Examination of the data in Table III shows that Magnolia rice grown on Crowley silt loam soil treated with 600 pounds of 0-9-6 fertilizer per acre and nitrogen in varying amounts from 12 to 54 pounds responded in growth and yield to all the fertilizer treatments. There was no significant difference in response, however, among the different amounts and kinds of fertilizer. Analysis of the data on the plot basis shows that there were no significant differences in the percentage of

TABLE III

Effect of Fertilizers on Protein Content and Yield of Magnolia Rice on Crowley Silt Loam, at the Rice Experiment Station, Crowley, Louisiana, 1949.

Pounds per A. $N-P_2O_5-K_2O$	Replicate	Moisture* %	Protein* %	Rough Rice lbs./A.
0-0-0	I	12.84	10.16	1532
0-0-0	II	13.53	10.47	1080
0-0-0	III	12.82	8.54	1066
	Mean		9.72	1226
0-18-12	I	13.44	9.86	2127
0-18-12	II	15.81	9.61	2000
0-18-12	III	13.04	10.06	1422
	Mean		9.84	1849
0-36-24	I	13.61	10.68	2085
0-36-24	II	14.22	10.49	1720
0-36-24	III	12.42	9.96	1377
	Mean		10.38	1727
0-54-36	I	15.64	10.47	1744
0-54-36	II	15.85	10.52	2000
0-54-36	III	15.98	10.50	1468
	Mean		10.49	1737
12-18-12	I	15.56	10.34	1915
12-18-12	II	13.46	9.53	1720
12-18-12	III	12.97	10.19	1489
	Mean		10.02	1708
24-36-24	I	16.05	10.48	1170
24-36-24	II	13.02	9.73	1720
24-36-24	III	15.39	10.64	1867
	Mean		10.28	1586
36-54-36	I	15.67	10.74	1191
36-54-36	II	15.74	9.95	1920
36-54-36	III	13.53	10.31	1889
	Mean		10.33	1666
18-18-12	I	12.66	10.01	2149
18-18-12	II	13.16	9.60	1220
18-18-12	III	13.86	9.88	1489
	Mean		9.83	1619
36-36-24	I	12.17	10.55	2064
36-36-24	II	13.34	9.82	1300
36-36-24	III	14.25	10.20	1156
	Mean		10.19	1507
54-54-36	I	15.63	9.99	1872
54-54-36	II	15.62	10.65	2200
54-54-36	III	15.95	10.28	1133
	Mean		10.31	1735

Percentage Protein is on a dry weight basis. Protein = 6.25 x N.
*Values are averages of duplicate determinations.
F value for percentage protein is 1.02 and is non-significant.

protein obtained from the different treatments including the checks or non-treated replicates. Statistical treatment of the data from each replicate indicates that the apparent differences in protein content were brought about by soil heterogeneity and not by the treatments. Further analysis of the data shows there is no significant correlation between the yield and the protein content. It should be noted, however, that the percentage of protein from the different treatments on Magnolia rice in this experiment is the highest recorded in this study, and that the natural soil conditions were more effective than fertilizer treatments on the influence of the percentage of protein in the rice. The rice in this experiment was planted late and did not mature until November. The late seasonal effects may have contributed to the inordinately high protein content and low yield of the Magnolia variety. Magnolia is normally an early variety. This further suggests that variety and varietal adaptations to season and soil are major factors in determining the percentage of protein in rice.

Data in Table IV indicate that the yield of Bluebonnet rice grown on Crowley silt loam at the Rice Experiment Station in 1950 was in-

TABLE IV

Effect of Fertilizers on Protein Content and Yield of Bluebonnet Rice on Crowley Silt Loam, at the Rice Experiment Station, Crowley, Louisiana, 1950.

Pounds per A. $N\text{-}P_2O_5\text{-}K_2O$	Moisture* %	Protein* %	Rough Rice lbs./A.
0-0-0	13.02	7.73	2365
0-0-20	12.44	8.06	2365
0-0-40	12.39	7.84	2511
0-20-0	12.38	7.95	2932
0-20-20	12.83	7.93	2981
0-20-40	11.98	7.64	3110
0-40-0	13.12	8.14	2738
0-40-20	13.13	7.84	2786
0-40-40	13.20	8.14	2786
20-0-0	12.18	7.96	2884
20-0-20	12.50	7.70	2333
20-0-40	12.55	7.68	2835
20-20-0	12.55	7.78	3143
20-20-20	11.94	7.88	3110
20-20-40	12.59	7.59	3353
20-40-0	12.48	7.86	3451
20-40-20	12.35	8.12	3564
20-40-40	12.46	7.83	3256
40-0-0	12.69	8 66	2543
40-0-20	11.61	7.84	2446
40-0-40	12.47	8 26	2745
40-20-0	11.91	7.65	2803
40-20-20	12.08	7.72	3645
40-20-40	12.14	7.56	3661
40-40-0	12.48	7 64	3094
40-40-20	12.00	8.01	3580
40-40-40	12.70	7.53	3094

Percentage protein is on a dry weight basis. Protein = 6.25 x N.
* Values are averages of duplicate determinations.

creased by nitrogen at both the 20 and 40 pounds per acre levels. Yield was further increased by addition of phosphate at both the 20 and 40 pound levels. Higher yields were obtained where both nitrogen and phosphate were used. There was little difference between the response to either nitrogen or phosphate at the 20 and 40 pound levels. Both nitrogen and phosphate were limiting factors on the yield of the rice. The inclusion of potash in the fertilizer mixture did not increase the yield of rice consistently. With nitrogen at the 40 pound level and phosphate at both the 20 and 40 pound levels the 20 pound level of potash did increase yields. With nitrogen at the 40 pound level and with no addition of phosphate the highest percentage protein was obtained. The relationship of a high nitrogen application and high protein with limited phosphate supply could not be measured statistically. It appears

TABLE V

Names and Origins of Varieties and Selections.

Sample No.	Variety or Selection
47	Zenith (Selection from Blue Rose)
37	Bluebonnet (Rexoro x Fortuna)
50	Dwarf Texas Patna (Selection of a natural cross on a dwarf mutation)
38	Selection No. 322A6-23 (Rexoro x Fortuna)
51	Lacrosse (Colussa-Blue Rose x Shoemed-Fortuna)
32	Arkrose (Caloro x Blue Rose)
31	Magnolia (Improved Blue Rose)
62	Nira
42	Selection No. 31-9-2-4 (Blue Rose-Rexoro x Blue Rose)
36	Century (Selection from Texas Patna x Rexoro-Supreme Blue Rose)
35	Fortuna
59	7/8 Rexoro-3-22 (Selection of Blue Rose-Rexoro x Rexoro x Rexoro)
58	Selection No. 4II-1-8 (Blue Rose-Rexoro x Rexoro)
61	Selection No. 3-72-3 (Rexoro x Purple Leaf)
39	Prelude (Improved Blue Rose x Fortuna)
43	Caloro
44	Improved Blue Rose
34	7/8 Rexoro-3-12 (Rexoro x Blue Rose-Rexoro)
60	Purple Leaf
33	Selection No. 6I-25-12 (Blue Rose-Rexoro x Blue Rose)
56	Rex-Nira (Selection from Rexoro x Nira)
46	Selection No. 3-72-3 (Rexoro x Purple Leaf)
41	B321B40-3 (Selection from Rexoro x Delitus)
52	Selection No. 4II-4-1-11 (Selection from Rexoro x F_1 of Rexoro x Blue Rose)
54	15/16 Rexoro-3-22 (Selection from Rexoro x Blue Rose-Rexoro)
45	Blue Rose 41 (Selection from Improved Blue Rose)
57	7/8 Rexoro-3-22
55	Texas Patna x Rexoro

that the high protein may have resulted from an excess of nitrogen due to limitation of growth from phosphate deficiency.

Statistical analysis of the data shows there are significant differences in percentage of protein among the different treatments. The differences are significant at both the 5 per cent and the 1 per cent levels. Because of the fact that the samples were composites from each treatment and not samples from each replicated plot the influence of soil heterogeneity could not be determined and the relationship between treatments could not be established statistically. A correlation coefficient was calculated for the association of yield and percentage protein. It was

TABLE VI

Protein Contents of Varieties and Selections of Rice from the Rice Experiment Station, Crowley, Louisiana, 1950.

Sample No.	Variety or Selection	Protein* Air-Dry Basis %	Moisture* %	Protein* Dry Wt. Basis %
47	Zenith	6.436	12.47	7.375
37	Bluebonnet	6.586	12.97	7.569
50	Dwarf Texas Patna	6.763	12.57	7.738
38	322A6-23	6.869	12.76	7.875
51	Lacrosse	6.983	13.00	7.975
32	Arkrose	7.106	13.15	8.181
31	Magnolia	7.119	13.05	8.188
62	Nira	7.169	12.74	8.213
42	3I-9-2-4	7.213	13.02	8.294
36	Century	7.316	12.97	8.400
35	Fortuna	7.325	13.12	8.431
59	7/8 Rexoro	7.375	12.98	8.475
58	4II-1-8	7.394	12.40	8.438
61	3-72-3	7.444	12.55	8.512
39	Prelude	7.488	12.98	8.606
43	Caloro	7.556	12.82	8.669
44	Improved Blue Rose	7.606	12.89	8.731
34	7/8 Rexoro-3-12	7.625	13.32	8.794
60	Purple Bran	7.663	13.03	8.781
33	6I-25-12	7.681	13.09	8.838
56	Rex-Nira	7.813	12.94	8.975
46	Rexoro x Purple Leaf	7.913	12.84	9.081
41	Rexoro-Delitus	7.931	13.26	9.144
52	4II-4-1-4	8.106	13.13	9.331
54	15/16 Rexoro-3-22	8.181	13.05	9.488
45	Blue Rose 41	8.594	12.66	9.838
57	7/8 Rexoro-3-22	8.625	13.13	9.931
55	Texas Patna x Rexoro	8.700	12.74	9.969
40	Rexoro	9.440	13.18	10.825

* Values given are averages of duplicate determinations.

found to be non-significant, thus showing there was no tendency for the two factors to vary together either in a negative or a positive direction.

Under the best field conditions and where nitrogen is a limiting factor approximately 2.4 pounds of fertilizer nitrogen must be applied

to produce 100 pounds of rough rice (51). Under the conditions of this experiment 40 pounds of nitrogen when applied with phosphate and potash increased the yield of rice approximately 1300 pounds. Only a 1600 pound increase could have been expected. This means that according to the theory of Russell (39), previously referred to, an increase in protein could not have been expected since yields were increasing so that

TABLE VII

The Distribution of Protein Nitrogen in the Varieties and Selections as Shown by Extraction with Different Solvents.

Sample No.*	H_2O-sol. Albumin[1] %	NaCl-sol. Globulin %	Alc.-sol. Prolamin[2] %	NaOH-sol. Glutelin[3] %	Insoluble %	Recovery %
47	9.72	14.06	1.46	35.23	38.55	106.06
37	11.16	7.70	2.07	37.97	41.10	109.96
50	9.96	6.98	1.18	38.68	43.22	94.73
38	9.98	13.96	1.86	35.25	38.97	100.73
51	11.57	15.67	1.80	38.53	31.52	100.07
32	10.11	12.92	0.76	43.31	32.91	104.06
31	9.35	13.29	0.69	43.90	32.79	102.72
62	7.05	12.90	1.26	32.57	46.25	105.51
42	7.91	14.61	1.83	35.74	39.93	100.87
36	9.00	8.81	1.60	34.33	46.27	106.61
35	9.48	12.02	1.75	36.24	40.51	102.56
59	7.79	13.37	1.58	35.51	41.77	94.56
58	7.77	15.43	1.63	32.91	42.77	94.64
61	8.26	12.23	1.85	31.50	46.17	96.00
39	8.36	13.13	2.14	46.65	29.74	107.43
43	9.84	13.48	2.67	49.64	24.48	106.40
44	8.73	14.26	1.42	41.43	34.18	104.63
34	6.77	8.80	2.02	39.39	43.03	103.63
60	7.69	13.77	1.09	38.70	38.76	107.87
33	9 84	13.16	1.62	41.91	33.48	102.91
56	8.10	15.88	1.05	39.66	34.73	97.31
46	6.91	13.27	1.69	34.98	43.12	95.80
41	8.49	11.57	1.58	42.78	35.59	102.44
52	6 07	12.17	1.58	42.57	37.63	105.77
54	6.78	13.60	1.31	39.36	38.96	101.18
45	9.01	10.43	0.70	46.80	33.06	99.16
57	6.58	12.82	1.14	41.39	38.07	100.04
55	7 24	14.24	2.24	38.33	37.98	98.95
40	6.15	11.67	2.27	45.61	35.36	102.55

All values in table are averages of duplicate determinations.

* Sample numbers correspond to those in Table V.

[1] Correlation with total protein r = −.695; [2]r = .776; [3]r = .399

there was not excessive accumulation of protein in the crop. Had the level of nitrogen applied been far in excess of what normally could have been used by the crop, consistent increases in the percentage of protein could have been expected from the application of excessive amounts of nitrogen. Such increases could be expected to be in the order of 10 to 20 per cent above normal.

Statistical analysis of the nitrogen contents of the more important varieties and selections of rice now being grown in Louisiana shows that there are significant differences in nitrogen or protein contents. Table VI reports the protein contents of samples of the varieties and selections used. The calculated F value is given in Table VIII. It shows that the differences in percentage of nitrogen among the varieties and selections are significant at both the 5 and 1 per cent probability levels. The least significant differences (L.S.D.) required for these two levels of probability are 0.057 and 0.077, respectively.

Examination of the data in Table VII reveals that the average percentage recovery of protein nitrogen for the extractions was 101.96 with a range of 94.56 to 109.96 per cent based on the total nitrogen determinations given in Table VI. Statistical analysis of the nitrogen distribution in the separate fractions shows that there are significant differences among the percentages of the total nitrogen occurring in each fraction for the different varieties and selections. The differences are significant at the 5 per cent and the 1 per cent probability levels as shown by the tabulated F values, Table VIII, determined by an analysis of variance.

TABLE VIII

Results of Statistical Analysis of the Protein Nitrogen Distribution in the Varieties and Selections as Shown by Extraction with Different Solvents.

	F	L.S.D. 5%	L.S.D. 1%	Correlation (r) with Total N
Total Nitrogen	29.01**	0.057	0.077	
Water Fraction	126.71**	1.19	1.61	−0.695**
5% NaCl Fraction	8.55**	2.21	2.97	0.078
70% Ethyl Alcohol Fraction	7.94**	0.48	0.64	0.776**
0.2% NaOH Fraction	17.85**	3.13	4.22	0.399*
Residue	31.52**	2.70	3.64	0.185

* Indicates significance at the 5 per cent probability level.
** Indicates significance at the 1 per cent probability level.

F value calculated to test the significance of differences among means.

L.S.D. Indicates the smallest difference necessary for significance at a particular probability level.

In Table VIII also are the L.S.D. values for the means of the nitrogen distribution in the solubility fractions of the protein.

Correlation coefficients were calculated to determine whether or not any of the solubility fractions were correlated with the total nitrogen. Since the total protein nitrogen content of the samples varied, the nitro-

gen in the different fractions was reported as a percentage of the total nitrogen content for each sample. By this means the nitrogen in the different fractions could be reported as a comparable basis. The correlation coefficients in Table VIII show that the water-soluble protein nitrogen is negatively associated with total protein nitrogen and that the alcohol-soluble protein nitrogen and dilute-alkali-soluble protein nitrogen are positively associated with the total protein nitrogen. The salt-soluble protein and insoluble protein nitrogen fractions are not correlated with total protein nitrogen. This means that with increasing total

TABLE IX

Varieties and Selections of Rice Arranged in the Order of Increasing Protein Nitrogen Content.

Sample No.	Variety or Selection	Total N %	H_2O-sol. % Tot. N	Alc.-sol. % Tot. N	NaOH-sol. % Tot. N
47	Zenith	1.033	9.72	1.46	35.23
37	Bluebonnet	1.054	11.16	2.07	37.97
50	Dwarf Texas Patna	1.082	9.96	1.18	38.68
38	322A6-23	1.099	9.98	1.86	35.25
51	Lacrosse	1.110	11.57	1.80	38.53
32	Arkrose	1.137	10.11	0.76	43.41
31	Magnolia	1.139	9.35	0.69	43.90
62	Nira	1.147	7.05	1.26	32.57
42	3I-9-2-4	1.154	7.91	1.83	35.74
36	Century	1.169	9.00	1.60	34.33
35	Fortuna	1.172	9.48	1.75	36.24
59	7/8 Rexoro	1.180	7.79	1.58	35.51
58	4II-1-8	1.183	7.77	1.63	32.91
61	3-72-3	1.191	8.26	1.85	31.50
39	Prelude	1.198	8.36	2.14	46.65
43	Caloro	1.209	9.84	2.67	49.64
44	Improved Blue Rose	1.217	8.73	1.42	41.43
34	7/8 Rexoro-3-12	1,220	6.77	2.02	39.39
60	Purple Bran	1.226	7.69	1.09	38.70
33	6I-25-12	1.229	9.84	1.62	41.91
56	Rex-Nira	1.250	8.10	1.65	39.66
46	Rexoro x Purple Leaf	1.266	6.91	1.69	34.98
41	Rexoro-Delitus	1.269	8.49	1.58	42.78
52	4II-4-1-4	1.297	6.07	1.58	42.57
54	15/16 Rexoro-3-22	1.309	6.78	1.31	39.36
45	Blue Rose 41	1.375	9.01	0.70	46.80
57	7/8 Rexoro-3-22	1.380	6.58	1.14	41.39
55	Texas Patna x Rexoro	1.392	7.24	2.24	38.33
40	Rexoro	1.504	6.15	2.27	45.61

nitrogen the water-soluble protein nitrogen decreases while the alcohol-soluble protein nitrogen and the dilute-alkali-soluble protein nitrogen increases. The salt-soluble protein nitrogen and the insoluble protein nitrogen fractions are not definitely related to the total nitrogen or protein content.

By arranging the varieties and selections in the increasing order of

their protein nitrogen contents, Table IX, and by considering that the varieties must differ from one another by 0.057 per cent protein nitrogen to be significantly different, the varieties and selections can be placed in low protein and high protein groups. These varieties and selections were grown in small plots under the same conditions. The soil was a uniform area of Crowley silt loam at the new Louisiana Rice Experiment Station. The soil is naturally fertile and was not highly fertilized. The differences may be considered as being inherently caused by variety. The Caloro variety with 1.209 per cent protein nitrogen occupies the median position. Zenith, Bluebonnet, Dwarf Texas Patna, 322A6-23, Lacrosse, Arkrose, Magnolia, Nira, 31-9-2-4, Century, and Fortuna are low in protein. Rex-Nira, Rexoro x Purple Leaf, Rexoro-Delitus, 4II-4-1-4, 15/16 Rexoro-3-22, Blue Rose 41, 7/8 Rexoro-3-22, Texas Patna x Rexoro, and Rexoro are in the high protein group. The varieties 7/8 Rexoro, 4II-I-8, 3-72-3, Prelude, Caloro, Improved Blue Rose, 7/8 Rexoro-3-12, Purple Bran, 6I-25-12 occupy a middle group. These are not significantly different from one another in protein nitrogen content, nor are they significantly different from the highest members of the low protein group or the lowest member of the high protein group.

It is particularly interesting to note that Rexoro and Blue Rose 41 are high protein rices and that certain crosses with Rexoro are high in protein. The combinations involving Rexoro and Blue Rose are high in protein. All Rexoro crosses, however, are not high in protein. Some of the outstanding crosses of Rexoro with Fortuna are low in protein. It appears that inheritance of high protein content is, as would be expected, complex and that whether or not a high protein variety can transmit the high protein characteristic depends also on the variety it is being crossed with. The genetic relationships are outside the scope of this study, but the data here does show that the protein contents of the commonly grown varieties vary as much as 50 per cent, which of itself indicates the great importance that the inherent characteristics of a variety play in determining the protein content. It is obvious that of the factors studied varietal inheritance has more to do with determining protein content than do soil conditions or fertilizer treatments.

SUMMARY AND CONCLUSIONS

A study has been made of the effects of fertilizer treatments on the protein content of rice grown on Crowley silt loam, of the variation in protein content of different varieties of rice, and of the distribution of protein fractions within the varieties and new selections now being grown at the Louisiana Rice Experiment Station. The fertilizer experiments were particularly concerned with the relationships between increasing amounts of fertilizer nitrogen and the protein nitrogen content of the rice. The protein content of the different varieties and new selections were determined on rice grown under uniform soil and seasonal conditions. The protein of each variety or selection was fractionated by the use of water, 5 per cent sodium chloride, 70 per cent ethyl alcohol, and 0.2 per cent sodium hydroxide.

Fertilizer treatments, particularly the mixtures containing nitrogen and phosphate, increased the yields of rice but they did not significantly increase the percentage of protein nitrogen.

Differences in the protein nitrogen due to soil heterogeneity were observed.

Factors which limit normal growth or yield, such as late planting and high application of nitrogen fertilizer with a limited available soil phosphorus supply, increased the percentage of protein nitrogen.

On the basis of all treatments there was no significant correlation between percentage protein nitrogen and yield of rice.

Statistical analysis of the protein distribution in the solubility fractions shows that there were differences among the percentages of the total nitrogen occurring in each fraction. Water-soluble protein was negatively correlated with total protein. Alcohol-soluble and alkali-soluble proteins were positively correlated with total protein. Salt-soluble and insoluble proteins were not definitely related to total protein.

The protein contents of the different varieties and selections varied between 6.44 and 9.44 per cent. Zenith occupies the low position, Caloro the median, and Rexoro the high.

Apparently, the inheritance of protein content is complex. Whether or not a high protein variety transmits the high protein characteristic depends also on the variety with which it is crossed. Varietal inheritance has more to do with determining protein content than do soil conditions or fertilizer treatments.

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